INFECTION OF BARLEY BY CLAVICEPS PURPUREA[1]

W. P. CAMPBELL[2]

Abstract

The development of infection of the ovary of Newal barley (*Hordeum vulgare* L.) by the ergot fungus, *Claviceps purpurea* (Fr.) Tul., was traced from the time of inoculation until formation of the sclerotia. Penetration took place within 24 hours near the base of the ovule. Initially, colonization was intercellular and confined to the ovary wall but by the fourth or fifth day the fungus began to move intracellularly, penetrating the ovule and also growing out onto the surface of the ovary to form a hymenium. Spores and honeydew were produced within five days of inoculation. The advancing hyphae were seldom found in stylar or stigmatic tissue and then only after the parasite was well established. Usually these structures are pushed upward to form a cap on the maturing sclerotium.

Introduction

Ergot, caused by *Claviceps purpurea* (Fr.) Tul., has long interested man, but it was not until 1711 that Geoffroy first suggested that it was a separate plant, a fungus growing on the ovaries of wheat. This view was confirmed by Münchhausen in 1764 and by several other writers in the latter part of the eighteenth century, finally to be brought forward again by DeCandolle (1815) as a new idea of his own. The above works have been cited by Barger (1) in his book *Ergot and Ergotism.*

It was not, however, until Tulasne (7) in 1853 studied this fungus that anyone seemed to have understood how it invaded, colonized, and replaced the ovaries in the infected florets. Kühn (4) in 1865 confirmed and extended the work of Tulasne and also grew the fungus on artificial medium. Engelke (2) in 1902 and Kirchhoff (3) in 1929 agreed that spores, either conidia or ascospores, land on the stigmata and germinate in the fluid secreted there. Engelke asserted that the fungus penetrates the stigma and grows down the conducting elements to the base of the ovule in much the same manner as does the pollen tube. From here it was supposed that the colonization of the ovary was in an apical direction because it already had been established by Kühn (4) that the sphacelia is always found first at the base of the ovary and grows upward from there. Kühn also stated that a heavy development of sphacelia is present at the base of the ovary while the internal tissues are still intact. Kirchhoff (3) agreed that the spores germinate on the stigma but stated that the fungus grows down over the external surface of the ovary to find a point of ingress at its base. He and Engelke thought that stigmata were of little importance as avenues of penetration, and Kirchhoff stated further that ingress is at the point of attachment of the ovary to the receptacle. From this area, he noted, the sphacelial strands grow upward through the

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Contribution No. 1682 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathology Laboratory, Edmonton, Alberta.

tissues of the ovary. Milovidov (5) wrote that, in artificial inoculation of rye ears with conidia, the spores germinated and formed dense mycelium, the hyphae of which attack the pistil and anthers and sometimes other flower parts. The hyphal mass presses on the inner cells of these organs and destroys them, using their contents as food, the debris being forced upward to form a cap on the maturing sclerotium. He also differentiated between sphacelial and sclerotial cells.

An analysis of the literature presents many contradictions in respect to the pathway of infection and the subsequent development of the pathogen in the host. The present work was undertaken to establish the histological pathology of ergot infection from the time of inoculation until maturation of the sclerotia.

Materials and Methods

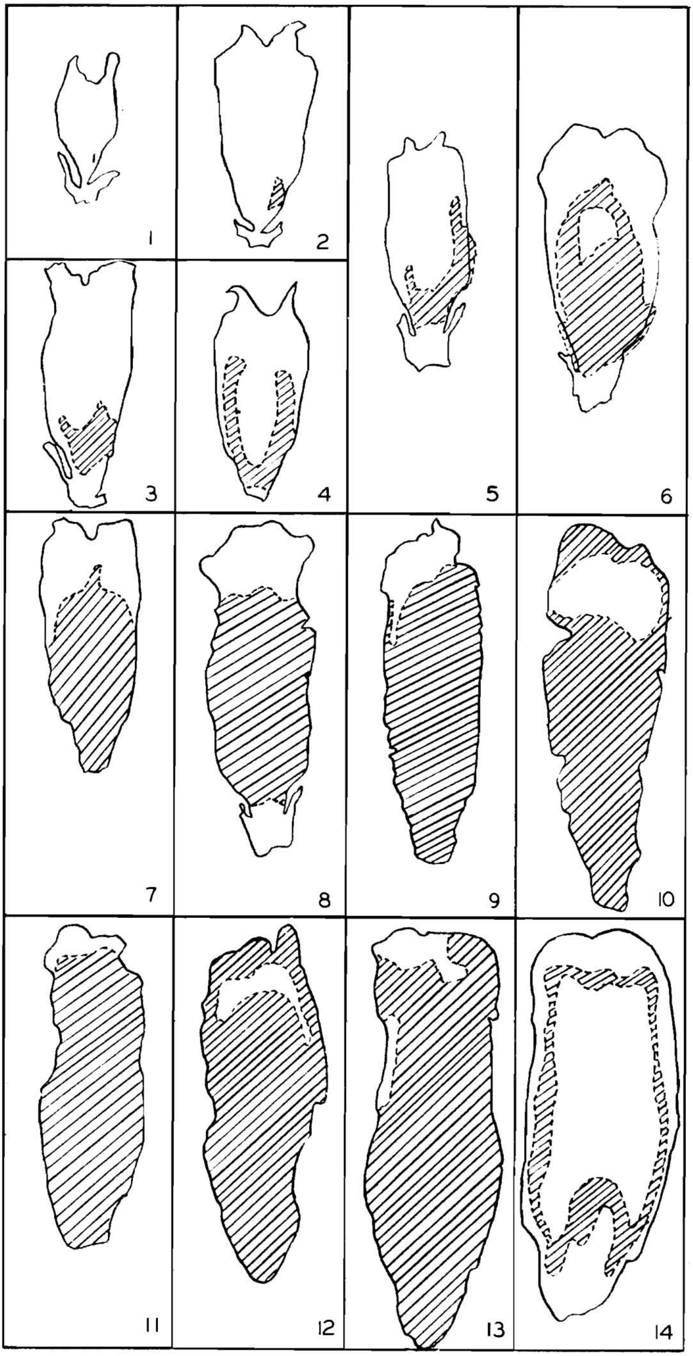
Newal barley grown in the greenhouse was inoculated with a suspension of *C. purpurea* conidia blown into the florets at flowering time with an atomizer. Florets were harvested at 24-hour intervals following inoculation and immediately fixed in a solution of the following formulation: 6 parts of isopropyl alcohol, 3 parts of propionic acid, and 1 part each of petroleum ether, acetone, and dioxane as outlined by Newcomer (6). The florets were then imbedded in paraffin, sectioned with a microtome, and stained by Conant's quadruple stain. The oldest material examined was collected 17 days after inoculation.

Results

Within 24 hours of inoculation the conidia germinated and produced a limited mycelium which penetrated the ovary near its base (Figs. 1 and 15). During the second and third days the mycelium was found passing through the ovary wall intercellularly (Figs. 16 and 17) until the integuments were reached (Fig. 18). While the integuments appeared to form a temporary barrier the fungus grew well in a generally upward direction through the ovary wall to entirely surround the ovule. By the fourth or fifth day the mycelium developed intracellularly as well as intercellularly (Figs. 19 and 20). At about this time hyphae began to erupt on the surface of the ovary (Fig. 21), there to produce the first conidia (Fig. 22), and the resistance of the integuments broke down to allow penetration and colonization of the ovule (Figs. 23 and 24). Cavities which became lined with a hymenial layer of conidiophores began to appear throughout the mycelial mass.

The fungus has not at any time been found to penetrate the style and stigma until at least a week after initial infection and then rarely. This penetration has been found only after these flower parts have already been overgrown by sphacelia from below.

The host tissue is not completely destroyed. There is usually a cap of uninvaded material from the stylar region on the top of the sclerotium or there may be islands of intact host tissue scattered throughout the ergot body.



FIGS. 1–14. Line drawings showing the progressive colonization of barley ovaries by *Claviceps purpurea*. Shading indicates area infected. FIG. 1. Ovary showing penetration near its base 24 hours after inoculation. FIGS. 2–13. Ovaries 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 15, and 17 days after inoculation, respectively. FIG. 14. An intact-appearing ovary which did not produce honeydew but which had a darkened, water-soaked appearance.

As the sclerotium begins to develop, starting always from the base, the conidial production ceases and the cavities within the fungus mass become filled with mycelium. There are two types of cells present in the mature sclerotium: relatively fine hyphal cells which penetrated and ramified through the ovary of the host; and large-diameter, irregular, barrel-like cells of later growth.

It has been noted that under certain circumstances, possibly late infection, the host plant produces seeds which seem normal. On sectioning, however, it is found that some of these contain a layer that appears to be intact but which has an irregular layer of *Claviceps* mycelium under it surrounding the endosperm (Fig. 14). An intact embryo is yet to be found in one of these seeds.

Downward development of the fungus from the infection court is limited, and it does not appear to penetrate very far into the receptacle. There is a rather definite line of demarcation between colonized and uncolonized host cells in the receptacle.

Figures 1 to 13 constitute a diagrammatic summary of the colonization of an ovary of barley and the development of a sclerotium.

Discussion

It has not been possible in this study to corroborate fully the findings of any one earlier author. The different stages of the infection phenomenon follow one another very rapidly. Unless observations are initiated within one day after inoculation and continued daily thereafter the various events may well be missed or misinterpreted. Failure to adhere to this procedure is probably the main reason for the apparent discrepencies of interpretation in the literature. If one were to start examining material after the production of honeydew had commenced it would be very easy to find support for the views of any or all previous workers. By that time the ovary was colonized and overrun by the fungus and even the stigma and style were occasionally found to be colonized, but this was only as a consequence of invasion from tissue below them.

Often we found sclerotia with a well-developed remnant of the seed on its apex, but we never found a seed with ergot tissue on its apex. Therefore it seems apparent that invasion and colonization of the ovary by the fungus

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FIG. 15. Initial penetration 24 hours after inoculation. (×500)

FIG. 16. Intercellular colonization 2 days after inoculation. (×1200)

FIG. 17. Intercellular colonization 3 days after inoculation. (×700)

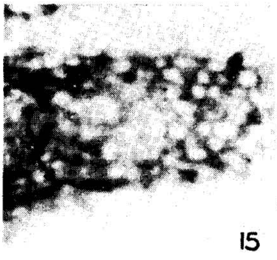
FIGS. 18 and 19. Advance of the mycelium temporarily halted at the integuments. (×700)

FIG. 20. Intercellular and intracellular colonization with attendant breakdown of the host tissue begins about the fourth day. (×700)

FIG. 21. Emergence of mycelium initiates the development of a fruiting layer on the surface of the ovary. (×1200)

FIG. 22. Well-developed fruiting layer on outer surface of ovary 5 days after inoculation. (×700)

FIGS. 23 and 24. Invasion of the ovule. Fifth and sixth days. (×700)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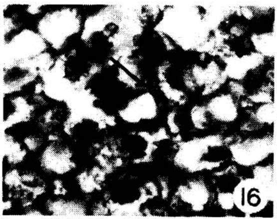
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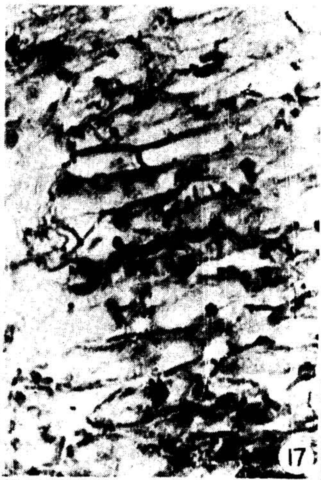
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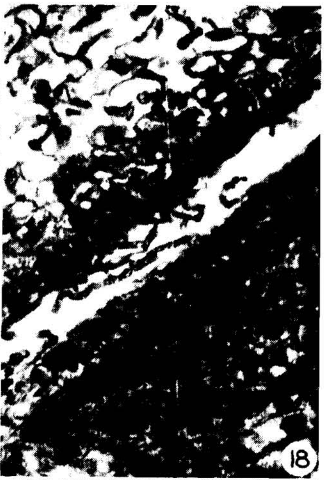
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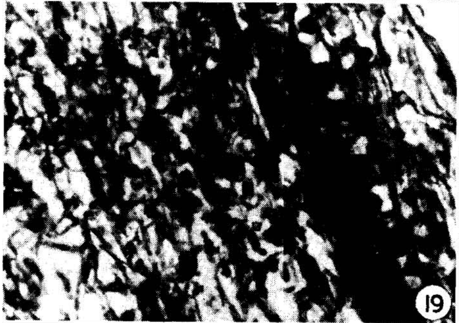
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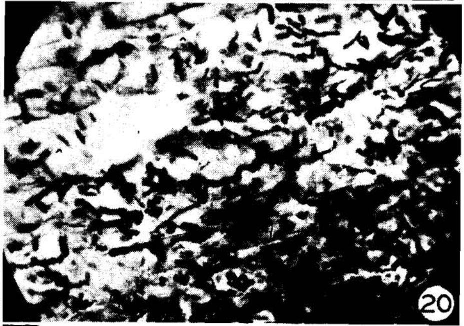
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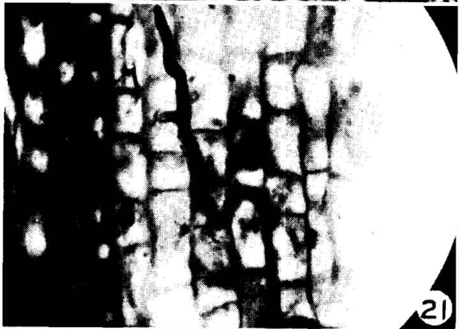
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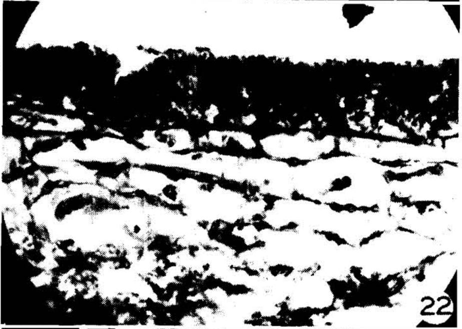
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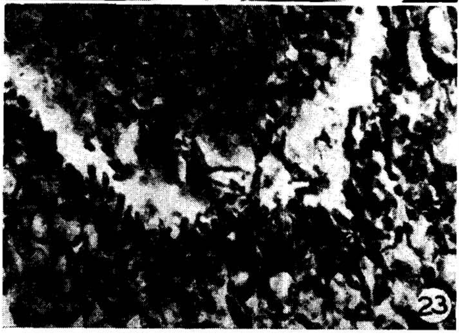
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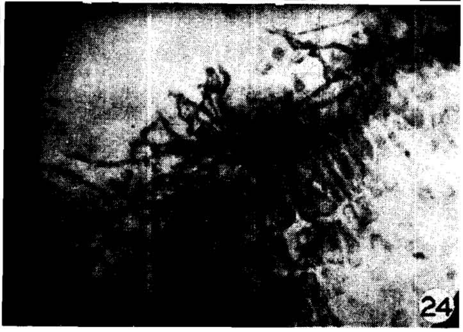
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must be from the bottom upward. The results of the present work, along with those of Kühn (4) and Kirchhoff (3), confirm this view. Unlike Kirchhoff, however, the author has not been able to find that spores germinate on the stigma and grow down over the surface of the ovary. Limited mycelial development was found on the exterior of the ovary and penetration appeared to be between the cells.

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